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16. ABSTRACT

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Effects of Ozone on Sporulation, Spore Germination, and Growth of *Fomes annosus*

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ABSTRACT

James, R. L., Cobb, F. W., Jr., and Parmeter, J. R., Jr. 1982. Effects of ozone on sporulation, spore germination, and growth of *Fomes annosus*. Phytopathology 72:1205-1208.

Effects of ozone (O_3) on certain cultural characteristics of *Fomes annosus* were investigated in exposure chamber studies. Growth rates of *F. annosus* decreased and conidial germ tubes were shorter and had fewer branches as O_3 dosages increased. *F. annosus* conidial production was very sensitive to O_3; very few conidia were produced when cultures were exposed to relatively low dosages (2,400 $\mu g/m^3$-hr). Conidial germination was decreased at the higher O_3 dosages, but spore germination was apparently stimulated at low dosages (184 $\mu g/m^3$-hr). Colonization of wood disks exposed to O_3 dosages of 16,870 $\mu g/m^3$-hr and greater was significantly less than that of nonexposed disks. However, O_3 dosages that can be expected under field conditions appear to have little potential effect upon those aspects of pathogen biology that would substantially affect epidemiology of annosus root rot.

Additional key words: air pollution, root disease.

Indirect effects of air pollution on the productivity of forest ecosystems may be as important as direct effects upon tree physiology and growth. For example, pollutants may influence the population dynamics of tree pests in ways that could cause dramatic increases in disease incidence. In the San Bernardino Mountains of southern California, oxidants apparently predispose pines to bark beetles (2). James et al (10,11) found that photochemical air pollution injury increases the susceptibility of ponderosa (*Pinus ponderosa* Laws.) and Jeffrey (*Pinus jeffreyi* Grev. and Balf.) pines to *Fomes annosus* (Fr.) Cke., an important root pathogen of conifers in California (1). Such increased susceptibility could lead to dramatic increases in disease incidence. On the other hand, pollutants may have direct effects upon the pathogen: reduced sporulation, spore germination, or growth.

Ozone (O_3), a major phytotoxic component of photochemical air pollution (16), directly affects pathogenicity of some fungi. Effects on growth (5,6), spore production (3,14,18), spore germination (4,7), and hyphal morphology (17) have been reported. Most of the previous work has involved foliar pathogens that were exposed to ambient pollutants directly.

The objective of this research was to investigate the effects of O_3 exposure under controlled conditions on the root pathogen *F. annosus*. Studies were made to determine effects on linear growth, conidial production, conidial germination, and colonization of freshly cut pine disks.

MATERIALS AND METHODS

Fumigation equipment. Two specially designed chambers constructed of clear, 6-mm-thick Plexiglas were used for all O_3 fumigations. Outside dimensions were 75 × 52.5 × 30 cm (volume = 0.11 m^3). PVC tubing (3.75 cm inside diameter) placed at each end provided for air intake and exhaust. Air baffles (20 cm × 12.5 cm) were erected 5 cm from the air intake and exhaust holes. Air mixing plates, with 400 0.9-cm-diameter holes, were placed inside each chamber 5 cm from each baffle. Tests indicated uniform concentrations throughout the chambers.

Air was drawn simultaneously through both chambers via flexible plastic tubing (6.25 cm in diameter) connected to a blower (Dayton Manufacturing Company, Dayton, OH 45429); flow rates in the two chambers were comparable. The flow rates, averaging 2.8 m^3/min, were determined at air intake manifolds by using an Anemotherm Air Meter (Anemostat Corp., New York, NY 10013). Filters of activated charcoal and glass wool to remove ambient O_3 and airborne fungal spores were placed inside the air-intake tubing of both chambers.

Temperature and relative humidity were monitored with a Honeywell Multipoint Potentiometer Recorder. Ozone was introduced in one chamber by illuminating a pen-ray ultraviolet lamp (Black Light Eastern Corp., Schenectady, New York, NY 12309) in the intake duct. Concentration was adjusted by manually moving the lamp relative to the air intake flow. Ozone was monitored with a Mast ozone analyzer (Mast Development Company, Ames, IA 50010) connected to a strip-chart recorder. The second chamber, with filtered air only, was used for control cultures.

Both fumigation chambers were placed inside a Percival plant growth lab in which relative humidity and temperature were controlled. The chambers were illuminated with incandescent light 12 hr daily.

Ozone effects on growth rate and conidial production. Four *F. annosus* isolates from the San Bernardino Mountains were used to study effects of O_3 on *F. annosus* linear growth rate and conidial production. The isolates were from Jeffrey pine (JLI and JPI), coulter pine (*P. coulteri* D. Don) (CI) and ponderosa pine (PPI).

Isolates were grown on potato-dextrose agar (PDA) for 10 days. Circular plugs of mycelium (6 mm in diameter) from the advancing colony margins were inoculated onto PDA in 9-cm petri dishes. These dishes were incubated at about 24 C for 72 hr before fumigation. Test dishes were divided into two 20-dish lots (one lot for each chamber) and colony diameter was measured (L_1). Dishes

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with their lids removed were randomly placed on racks in chambers.

Cultures were fumigated 9 hr daily for 3 days at four O_3 concentrations (98, 196, 431, and 882 $\mu g/m^3$ = 0.05, 0.10, 0.22, and 0.45 ppm). Facilities necessitated consecutive rather than concurrent exposures at the four concentrations. Thus, a set of control cultures was included with each set of exposed cultures. Temperature in both chambers was 24.0 ± 1 C, and relative humidity was 96 ± 4%.

Colony diameter (L_2) was recorded after fumigation, and growth was calculated for the exposure period as $L_2 - L_1$. Spores in each dish were harvested by adding 5 ml of distilled water and agitating conidiophores with a fine, camel's hair brush. Spore counts were made with a standard hemacytometer (Levy-Hausser counting chamber).

Statistical analysis consisted of one-way analysis of variance, which compared the controls with O_3-fumigated cultures. Linear growth rate and conidial production were expressed as percentages of the controls when comparisons were made among exposure lengths within concentrations.

Ozone effects on conidial germination. Two of the isolates (JLI, PPI) used in the growth and sporulation studies described above were also used in an experiment to test O_3 effects on conidial germination. Conidia were harvested from 10-day-old cultures grown on PDA by adding 5 ml of sterile distilled water and agitating with the camel's hair brush. Suspensions were diluted to about 10,000 conidia per milliliter water. The spore suspension (0.1 ml) was added to the center of each 9-cm-diameter water agar test dish and spread evenly over the agar surface with a flame-sterilized glass rod.

Test dishes were grouped in two 10-dish lots for each isolate and placed randomly in chambers. Spores were fumigated for 1, 2, 4, or 8 hr at 196, 431, or 882 $\mu g/m^3$ O_3 (0.10, 0.22, and 0.45 ppm, respectively). After exposure, spores were incubated in the dark for 24 hr at about 22 C. Germination percentages were determined by checking for germ tube emergence under the compound microscope. One hundred conidia per dish were examined; those with an emerging germ tube were considered germinated.

Germination percentages were transformed using the arc sine square root transformation to remove dependence of variances on means. The transformed germination percentages for each group of exposed colonies was compared to its control group by using the "t" test. The means of the differences between the mean of the control colonies and each exposed colony were compared for the four different exposure times at each O_3 concentration. The Studentized range test (Student-Newman-Keuls Test [12]) was used to detect significant differences among exposure times for a given concentration.

Two *F. annosus* isolates were used in another experiment to test O_3 effects on conidial germ tube growth. These were JLI, used in the previous experiment, and HBII from an infected Jeffrey pine (San Bernardino Mountains). For this experiment, conidia were obtained from 10-day-old cultures and placed on water agar in dishes as described in the previous experiment. Ten dishes per isolate per chamber were fumigated for 12 hr at 176.4, 352.8, 490.0, or 1,411.2 $\mu g/m^3$ O_3 (0.09, 0.18, 0.25, and 0.72 ppm).

Following fumigation, dishes were immediately checked for conidial germination. Twenty-five germinating conidia per dish were chosen for germ tube length measurement. Percentages of branched germ tubes were also recorded.

Ozone effects on wood colonization. Two *F. annosus* isolates, JLI and HBII, were used to evaluate effects of O_3 on the colonization of wood disks. Disks were cut from ponderosa pine stem sections that had been swabbed with 95% ethanol to reduce contamination. They ranged from 65 to 75 cm in diameter and were ~1.0–1.5 cm thick. Disks were kept frozen until inoculated.

Conidial suspensions were prepared from 14-day-old *F. annosus* cultures, as previously described. Suspensions were diluted to about 40,000 conidia per milliliter of water.

Each pine disk was inoculated uniformly with 0.5 ml of the spore suspension applied with a fine mist atomizer. Twenty disks per isolate were inoculated for each O_3 fumigation trial. Inoculated disks were divided into two equal lots and placed randomly in the control and O_3-exposure chambers.

Disks were fumigated for 9 hr daily 7 days at 117.6, 196, 215.6, or 529.2 $\mu g/m^3$ O_3 (0.06, 0.10, 0.11, and 0.27 ppm). The extent of *F. annosus* colonization, defined by presence of the *Oedocephalum* state, was then determined by examination under the dissecting microscope. Colonization was outlined with a felt pen, and the percentage of surface area covered was determined with a dot grid overlay (four dots per square centimeter).

RESULTS

Ozone effects on growth rate and conidial production. As O_3 dosage increased, linear growth rate and conidial production of *F. annosus* decreased (Table 1). Significant reductions in growth rate of exposed cultures, compared to controls, occurred at O_3 concentrations of 196 $\mu g/m^3$ (0.10 ppm) or greater. Differences in conidial production occurred at all O_3 concentrations above background. However, cultures removed from O_3 exposure resumed approximately normal sporulation and growth.

Ozone effects on conidial germination. The percentage germination of spores exposed to O_3 was generally significantly less than that of the controls (Table 2). However, germination of spores of both test isolates apparently was stimulated by the lowest O_3

TABLE 1. Influence of ozone dosages on linear growth and conidial production of four *Fomes annosus* isolates

Isolate	Ozone conc.[a] (ppm)	Ozone dosage ($\mu g/m^3$-hr)	Growth rate (mm/hr)			Conidial production (no./mm^2)		
			Exposed	Control	% Of control	Exposed	Control	% Of control
JLI	0.045	2,401	0.45	0.58	77.6[b]	10.1	62.0	16.3
	0.10	5,634	0.04	0.39	10.3	1.1	92.1	1.2
	0.22	11,477	0.06	0.50	12.0	0.0	76.3	0.0
	0.45	23,814	0.02	0.32	7.5	0.0	185.8	0.0
JPI	0.050	2,646	0.37	0.49	75.5[b]	16.1	110.6	14.6
	0.10	6,350	0.09	0.46	19.6	8.1	97.4	8.3
	0.22	11,477	0.06	0.53	11.3	4.6	78.1	5.0
	0.45	25,402	0.02	0.43	4.6	0.0	89.6	0.0
CI	0.045	2,401	0.53	0.61	86.8[b]	6.2	60.0	10.3
	0.10	6,350	0.20	0.46	42.6	14.4	168.3	8.6
	0.22	13,230	0.07	0.38	18.9	4.6	128.6	3.6
	0.45	23,814	0.05	0.33	14.8	0.0	143.1	0.0
PPI	0.050	2,646	0.23	0.28	82.1[b]	64.3	187.4	34.3
	0.10	5,634	0.08	0.26	30.8	55.9	200.0	28.0
	0.22	13,230	0.08	0.42	19.0	12.6	167.3	7.5
	0.45	25,402	0.02	0.36	5.5	0.0	159.4	0.0

[a] Cultures were fumigated 9 hr daily for 3 days (27 hr total). In each case there were 20 exposed and 20 control colonies.
[b] No significant difference ($P = 0.05$) between exposed and control cultures. All other such comparisons were significantly different.

TABLE 2. Influence of ozone exposure on conidial germinations of two *Fomes annosus* isolates

Exposure time (hr) in chambers	Ozone conc. (ppm)	Total ozone dosage ($\mu g/m^3$-hr)	Isolate JL1			Isolate PP1		
			Germination[a] (%)		Mean[b] difference from control	Germination[a] (%)		Mean[b] difference from control
			Exposed	Control		Exposed	Control	
1	0.10	184	93.4*	90.3	−0.118 A	94.1*	91.0	−0.137 A
2	0.10	397	85.4	89.6	0.119 B	80.2*	91.8	0.337 C
4	0.10	749	86.4*	94.6	0.282 C	85.2*	90.6	0.154 B
8	0.10	1,570	83.8*	91.7	0.240 C	81.9*	84.4	0.210 BC
1	0.22	445	87.1*	92.2	0.164 D	75.6	73.1	−0.054 D
2	0.22	932	85.7*	91.7	0.188 D	68.3	72.4	0.087 D
4	0.22	1,770	83.5*	93.4	0.327 E	55.6*	72.1	0.345 E
8	0.22	4,170	78.9*	90.2	0.314 E	40.9*	71.4	0.632 F
1	0.45	888	82.1*	89.3	0.203 F	66.9*	88.1	0.520 G
2	0.45	2,060	79.7*	91.5	0.342 G	56.6*	89.6	0.782 H
4	0.45	4,300	57.8*	90.1	0.774 H	54.3*	90.2	0.848 I
8	0.45	9,540	29.1*	88.8	1.320 I	35.2*	88.2	1.170 J

[a] All values are means of percent germination for 10 colonies. Exposed means labeled * were significantly different from controls ($P = 0.05$).
[b] Means of differences between percent germination of each exposed colony and the mean percent germination of controls. Values are in radians from the arc sine square root transformation. Within each ozone concentration, means with the same capital letter are not significantly different ($P = 0.05$) using the Studentized range test (Student-Newman-Keuls test).

dosage (0.1 ppm for 1 hr) and was unaffected by other low dosages. As the O_3 dosages increased, spore germination decreased to approximately one-third of the controls at the highest dosage (0.45 ppm for 8 hr).

Data from the second study in which both conidial germination and germ tube growth were measured again showed that as dosage increased, conidial germination decreased, at 16,993 $\mu g/m^3$ O_3 (0.72 ppm for 12 hr), no conidia germinated (Table 3). Average germ tube length decreased significantly with increasing O_3 dosage. Germ tubes were about half the normal length and much less branched when exposed to concentrations as low as 0.09 ppm.

Ozone effects on wood colonization. Colonization of wood disks while being exposed to O_3 was significantly less than that of the controls at concentrations as low as 0.10 ppm in the case of *F. annosus* isolate JL1 and 0.11 ppm in the case of HBI1 (Table 4). As O_3 dosage increased, disk colonization by *F. annosus* decreased. However, the reduction was only 20 and 35% for the two isolates even at the highest dosage (31,928 $\mu g/m^3$-hr) tested.

TABLE 3. Influence of ozone dosages on conidial germination and germ tube growth of *Fomes annosus* after 12 hr of exposure

Isolate	Ozone conc. (ppm)	Ozone dosage ($\mu g/m^3$-hr)	Germination (%)	Avg. germ tube length[a] (mm)	Conf. limit	Branched germ tubes (%)
JL1	0	0	41.8	0.077	± 0.0063	20.0
	0.09	2,205	23.5	0.039	± 0.0025	7.0
	0.18	4,145	4.0	0.038	± 0.0031	11.2
	0.25	5,885	2.2	0.022	± 0.0022	4.0
	0.72	16,993	0	...	...	...
HBI1	0	0	9.1	0.079	± 0.0058	13.4
	0.09	2,205	21.0	0.041	± 0.0025	2.6
	0.18	4,145	4.8	0.036	± 0.0031	5.6
	0.25	5,885	3.4	0.029	± 0.0032	4.0
	0.72	16,993	0	...	...	...

DISCUSSION

Ozone, a major phytotoxic component of photochemical air pollution, may affect disease epidemiology through various direct effects upon the pathogen. Other investigators (6,13,20) have reported that growth of such fungi as *Colletotrichum lindemuthianum* and *Erysiphe graminis* was reduced while cultures were being exposed to O_3. Low dosages of O_3 have reduced sporulation by *Puccinia graminis* f. sp. *tritici* (5) and *C. lindemuthianum* (20). On the other hand, O_3 has been reported to stimulate sporulation of *Alternaria oleraceae* (20), *A. solani* (18), and *Mycosphaerella citrullina* (18).

Percentage spore germination is usually inversely related to O_3 dosage (7,19). Small, hyaline spores such as those of *Fusarium oxysporum*, *Colletotrichum lagenarium*, *Verticillium albo-atrum*, and *V. dahliae* are inhibited more by O_3 than large, pigmented spores of species like *Chaetomium* sp., *Stemphylium sarcinaeforme*, *S. loti*, and *Alternaria* spp. (4,7). Small dosages may actually stimulate spore germination of some fungi (7,15).

Our results show that direct exposure of *F. annosus* to O_3 reduces growth of hyphae and germ tubes, inhibits conidial production, and reduces conidial germination and colonization of wood disks. However, even when *F. annosus* cultures were exposed to O_3 concentrations of about 0.05 ppm, near the maximum expected under field conditions, for 9 hr daily, colony growth was reduced only 20–25%. Since vegetative growth of *F. annosus* occurs primarily in plant tissues, the small direct effects of O_3 and other similar pollutants on such growth may have little, if any, influence on disease epidemiology.

Our results also indicate that germination of *F. annosus* conidia is not substantially affected at dosages that more realistically reflect field conditions. For example, at 0.10 ppm for 8 hr (1,574 $\mu g/m^3$-hr) germination was reduced less than 10%, and at 0.10 ppm for 1 hr (184 $\mu g/m^3$-hr) it actually increased. Germ tube elongation and branching appeared to be as sensitive as germination; at 0.09 ppm for 12 hr (2,204 $\mu g/m^3$-hr), both were reduced about 50%. However, at the lesser dosages (>0.05 ppm) expected in the field, especially when spore germination would be occurring, influence of reduced germ tube elongation on overall epidemiology is probably minimal. The results of the experiment on pine disk colonization tend to support this conclusion. When pine disks used to simulate freshly cut stump surfaces were inoculated with conidia and exposed to a concentration of 0.06 ppm O_3, 9 hr per day for 7 days, there was no reduction in colonization. Even when the dosage was approximately doubled, reduction in colonization was only 18–26%.

The greatest effect of O_3 detected in these studies was upon conidial production. At dosages as low as 0.05 ppm, the numbers of conidia produced were reduced 65–90%. If such reductions in inoculum occur frequently in the forest, the effect upon disease epidemiology could be significant. However, basidiospores are thought to be the major inoculum for stump infection, and we do not know yet whether the effect of O_3 on basidiospore (and basidiocarp) production will parallel that on conidial production. We have found no relationship between deposition rate of *F. annosus* basidiospores and general level of air pollution in the San Bernardino Mountains (8).

TABLE 4. Effect of ozone dosages on the colonization of pine disk by two isolates of *Fomes annosus* after exposure for 9 hr per day for 7 days

Isolate	Ozone concentration (ppm)	Ozone dosage ($\mu g/m^3$-hr)	Disk colonization (%)		Percent of control
			Control	O_3-Exposed[a]	
JLI	0.06	8,736	71	76	107
	0.10	12,595	76	59**	78
	0.11	16,868	80	60**	75
	0.27	31,928	84	55**	65
HBII	0.06	8,736	79	74	94
	0.10	12,595	78	68	87
	0.11	16,868	76	62**	82
	0.27	31,928	71	57**	80

[a]Means followed by ** are significantly different ($P = 0.01$) from the controls based on one-way analysis of variance.

Timing of exposure is an important factor in evaluation of oxidant effects upon inoculum production and subsequent dispersal and infection of stumps. *F. annosus* spore production apparently is favored by moderate temperatures and moist conditions. Hence, periods of maximum spore production would tend to occur at times when photochemical oxidants are not at their maximum concentrations. Furthermore, studies in the San Bernardino Mountains (8) and elsewhere have shown that maximum spore release occurs at night, a time when oxidant concentration is usually low.

The studies reported here are not concerned with genetic adaptability or possible changes in virulence in response to air pollutants. However, the data do indicate that there are differences in the sensitivity of *F. annosus* isolates to O_3. For example, the linear growth rate of isolate CI appeared to be affected less than that of the other three isolates, whereas conidial production of isolate PPI was affected less. With respect to conidial germination, isolate JLI was more tolerant than PPI. No evidence of adaptation of *F. annosus* to O_3 was found in a study of isolates from areas with a history of exposure to moderately high levels of photochemical air pollutants and isolates from areas with low levels of pollution (9). There were large differences in infection and colonization rates among isolates, but differences appeared to be unrelated to isolate source.

On the basis of the results reported here, we conclude that there is little or no direct effect of O_3 on *F. annosus* that would substantially affect its epidemiology. Our studies do not, however, rule out the possibility that basidiospore production and germination may be significantly affected by ambient O_3 concentrations.

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